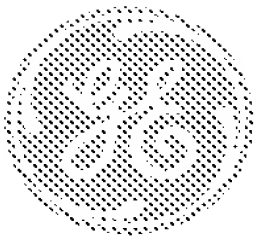
GE Healthcare

illustra QuickPrep mRNA Purification Kit

Product booklet

Code: 27-9254-01



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1. Legal

Product use restriction

The illustra™ **QuickPrep™ mRNA Purification Kit** has been designed, developed, and sold for **research purposes only**. It is suitable for ***in vitro* uses only**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the **QuickPrep mRNA Purification Kit** for a specific application range as the performance characteristic of this material has not been verified to a specific organism.

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Extraction Buffer contains guanidine thiocyanate. Wear gloves and safety glasses.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

All kit components should be stored at 2-8°C.

2.3. Expiry

For expiry date, please refer to outer packaging label.

3. Components

3.1. Kit contents

Table 3.1. QuickPrep mRNA Purification Kit contents

Pack Size: 4 preps Cat. No.: 27-9254-01	Volume	Quantity
Oligo (dT)-Cellulose columns	~ 4 ml resin each	4
Extraction Buffer	3.0 ml	4 vials
High Salt Buffer	10.0 ml	4 vials
Low Salt Buffer	7.0 ml	4 vials
Elution Buffer	6.0 ml	4 vials
Sample Buffer	500 µl	2 vials
Glycogen Solution	100 µl	2 vials
Potassium Acetate Solution	200 µl	2 vials

3.2. Reagents and materials to be supplied by user

All plasticware or glassware which may come into contact with the sample should be RNase-free (5).

- Mechanical or manual tissue homogenizer (optional)
- 15 ml centrifuge tubes
- 1.5 ml screw-top microcentrifuge tubes
- 95-100% ethanol
- DEPC treated or RNase-free water

4. Description

4.1. The basic principle

The illustra **QuickPrep mRNA Purification Kit** is designed for the direct isolation of polyadenylated RNA from eukaryotic cells or tissues, bypassing the need for intermediate purification of total RNA. Each purification can be performed in approximately 1 hour starting from as little as one cell or as much as 0.5 g of tissue. The mRNA isolated with the kit can be used in numerous applications, such as cDNA synthesis, Northern analysis, *in vitro* translation and PCR (1). Sufficient reagents are provided for four mRNA purifications.

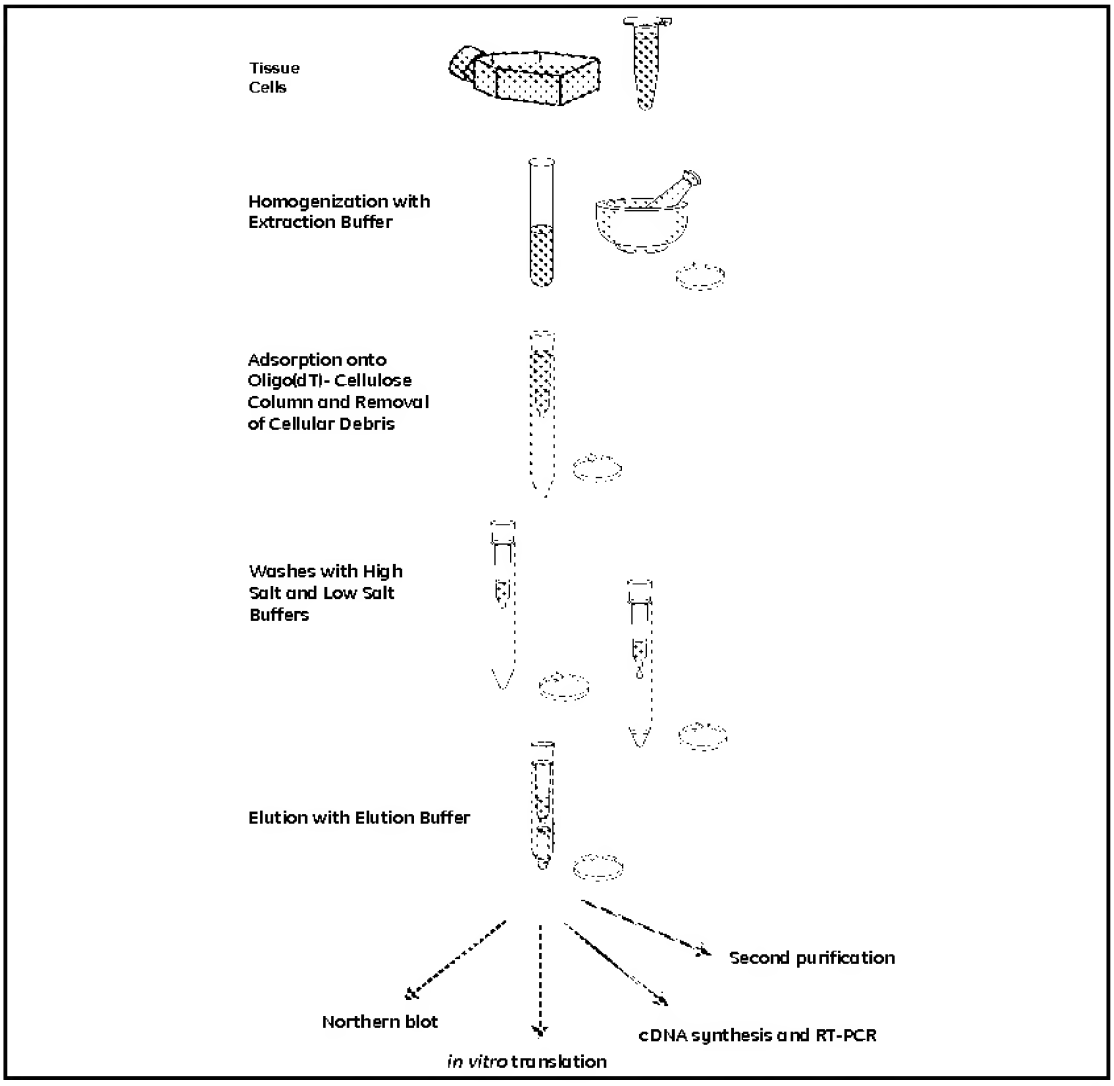


Figure 4.1. Overview of the **QuickPrep mRNA Purification Kit** procedure

QuickPrep mRNA Purification Kit is designed for the rapid isolation of mRNA from eukaryotic cells or tissues without the need for intermediate purification of total RNA. Each purification can be completed in as little as 1 hour depending on the purity of mRNA desired, the amount of starting material, and the mRNA content of this material. The kit is well suited for the isolation of mRNA from cells or tissues which are available in limited quantities because it eliminates losses associated with intermediate purification of total RNA. We have isolated mRNA from as little as one cell and as much as 0.5 g of tissue.

QuickPrep mRNA Purification Kit combines the disruptive and protective properties of guanidine thiocyanate (GTC, (2) and (3)) with the speed and selectivity of Oligo (dT)-Cellulose chromatography in a spin column format pioneered by GE Healthcare (4). The protocol is outlined in Section 7 and briefly described below.

Initially, the tissue is extracted by homogenization in a buffered solution containing a high concentration of GTC. This ensures the rapid inactivation of endogenous RNase activity and the dissociation of cellular components from the mRNA. The extract is then diluted with Elution Buffer to optimally reduce the GTC concentration to allow efficient hydrogen bonding between poly(A) tracts on mRNA molecules and Oligo (dT) attached to cellulose, but high enough to maintain inhibition of RNases. As an added benefit, a number of proteins precipitate, allowing their removal easily by centrifugation. After a brief second homogenization, the extract is clarified by centrifugation, the supernatant is poured into an Oligo (dT)-Cellulose Spin Column, and the polyadenylated fraction is allowed to bind over a short period of time with gentle mixing. The column is subjected to a low speed centrifugation and the liquid containing the unbound material is decanted. The resin is washed sequentially with High Salt

and then Low Salt Buffer. Finally, the sample is eluted from the resin with prewarmed Elution Buffer.

4.2. Kit specifications

Each spin column has the capacity to bind up to 25 µg of polyadenylated RNA. The purity of mRNA relative to other biomolecules is dependent on the number of cells extracted. If fewer than 10^7 cells are extracted, the mRNA isolated will have a purity of greater than 90%; with progressively larger tissue samples, the purity may gradually drop to ~ 50%.

The RNA isolated with the kit is essentially free of DNA and protein contamination. It should be of sufficient purity for most applications. If further purification of the sample is desired, it may be subjected to a second spin column purification as described in Section 7.3.

The use of a second column will consume a second set of reagents, thus decreasing the total number of purifications possible with the kit. The use of a second column may not be advisable when purifying mRNA from small amounts of cells because it may reduce the final yield below useful levels for some applications.

Table 4.1. QuickPrep mRNA Purification Kit Technical Specifications at a Glance

	Tissue or Cells
Sample size	< 0.5 g of tissue or < 5×10^7 cells
Elution volume	750 µl
Binding capacity	25 µg poly(A) RNA
Relative amount of poly(A) RNA in eluate (Purity)	≥ 90% with 10^7 or fewer cells
Time/Prep	1 hour/purification

The kit contains both potassium acetate and glycogen solutions for precipitation of mRNA in cases where it is not sufficiently concentrated for use directly in the subsequent selected application.

5. Preparation of working solutions

Extraction Buffer and Potassium Acetate solutions are irritants and should be handled with care.

Avoid ribonuclease contamination, especially those present on the skin, by wearing clean gloves at all times and using RNase-free or DEPC-treated glassware and plasticware. Also, prepare buffers with RNase-free water.

5.1. 0.1% DEPC treated water

Prepare a 0.1% (v/v) solution of **Diethyl Pyrocarbonate (DEPC)** in distilled water, shake vigorously, and allow to stand overnight at room temperature. Autoclave the solution on the following day with the cap loosened. Commercially available RNase-free water may be used instead of DEPC-treated water in this protocol

5.2. TE buffer

10 mM **Tris-HCl, pH 7.4**, 1 mM **EDTA**, prepared with DEPC-treated or RNase-free water. Autoclave the buffer after the addition of the Tris. Commercially available **TE (pH 7.4)** with this formulation may also be used.

6. Handling, preparation, and storage of starting materials


RNA is not protected against degradation until the sample is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore, it is important that samples are flash frozen in liquid nitrogen immediately and stored at -70°C in a stabilizing agent or processed as soon as possible.


A centrifuge with a swinging bucket rotor and appropriate buckets capable of reaching 4 000–4 500 × *g* is required.


Wear gloves at all times during the preparation. Change gloves frequently.

Approximately 20–30 minutes before sample extraction, remove the Extraction Buffer from 4°C storage and place at 37°C. Shake the bottle occasionally until all the crystalline material is dissolved. If the crystalline material persists, place the bottle at 55°C and shake occasionally. If it is difficult to get the final crystals into solution, simply allow the crystalline material to settle and pipette the solution away from the crystals. This will not result in any deleterious effects on buffer performance. Cool Extraction Buffer to room temperature before use.

Tissues (up to 0.5 g)

Place the tissue in a chilled homogenizer (either manual or mechanical) and add **1.5 ml of Extraction Buffer**. Homogenize the tissue until it is a uniform suspension. Avoid the generation of excess heat or foam.

Cultured cells (up to 5×10^7 cells)

To extract cells grown as a monolayer:

Drain the culture medium from the cells in one 75 cm^2 flask, then add **1.5 ml of Extraction Buffer** directly onto the cells. Swirl the buffer over the monolayer to assure complete cell lysis. If desired, this suspension can be transferred onto an additional monolayer to lyse these cells. Alternatively, free adherent cells from anchorage by standard methodology and suspend them in a small volume of isotonic buffer. Add **1.5 ml chilled Extraction Buffer**.

To extract cells grown in suspension:

Place an aliquot of cell suspension containing up to 5×10^7 cells in a microcentrifuge tube and pellet by centrifugation. Remove the supernatant. Add **1.5 ml of Extraction Buffer** to the pelleted cells. Vortex gently for 1 min.

7. Protocols


Steps 1 and 2 must be performed without pause, from disruption of cells or tissue to elution of the poly(A) RNA.
Make sure that all materials are ready before starting the procedure.
Work at room temperature except where specifically directed otherwise.

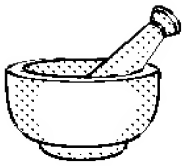
7.1. Isolation of mRNA


The instructions which follow are for a single column purification. If a second column purification step is desired, refer to Section 7.3.


The columns may become slippery if Extraction Buffer is spilled on the outside surface, so exercise caution when handling them.

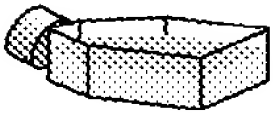
1. Homogenization of sample

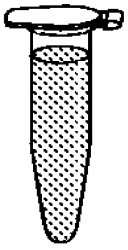
Disrupt up to **0.5 g tissue** in a chilled homogenizer using **1.5 ml Extraction Buffer**.



Disrupt sample

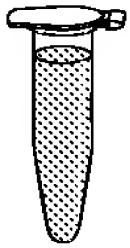
Disrupt up to **5 × 10^7 cells** by direct addition of **1.5 ml Extraction Buffer** to a drained culture monolayer or to a cell pellet collected in a microcentrifuge tube by centrifugation.



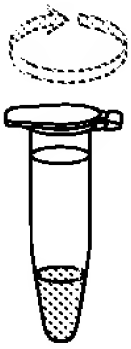


(See Section 6 for additional method details).

Add **1.5 ml of Extraction Buffer** to the pelleted cells.

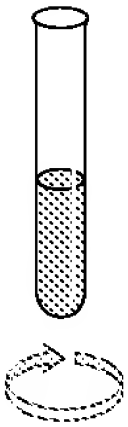


+ 1.5 ml chilled Extraction Buffer



2. Cell Lysis

Dilute sample by adding **3 ml of Elution Buffer** and mix thoroughly. Homogenize briefly. Transfer the homogenate to a sterile polypropylene centrifuge tube.



+ 3 ml
Elution Buffer

Mix

Spin 5–10 min
12 000 × *g*

Place the remaining **Elution Buffer** at **65°C** until needed in step 8.

Centrifuge tubes for **5–10 min at 12 000 × *g* (10 000 rpm)**.

3. Prepare the Oligo (dT)-Cellulose Spin Column

Invert an **Oligo (dT)-Cellulose Spin Column** (provided) **several times** to resuspend resin. Remove both top and bottom closures, place the column in a 15 ml centrifuge tube. Centrifuge for **2 min at 350 × *g***.

The widest portion of the column, at the top, does not have to fit inside the 15 ml tube.

Remove column from centrifuge tube and discard the flow-through. Replace the bottom closure on the drained column and place it upright in a rack.

Mix Spin Column Resin

Remove top & bottom closures

Spin 2 min 350 × *g*

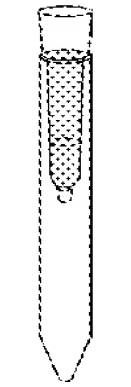
Discard flow-through

4. Removal of cellular debris

Apply **4 ml of supernatant** from step 2 onto the **Oligo (dT)-Cellulose Spin Column**. Avoid disturbing the pelleted material.

+ 4 ml supernatant to resin

Replace the top closure on column and **mix by inversion** for **3 min** or by placing tubes on a rocking table or similar device.



Mix by inversion 3 min

Leaving both closures securely on, place the column in a 15 ml centrifuge tube. Centrifuge at **350 × *g* for 2 min** to separate the resin from the suspension.

Spin 2 min 350 × *g*

Remove the top closure, decant the cell debris containing supernatant and discard it. Avoid disturbing the resin.

Discard supernatant

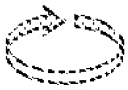
5. Washes

High Salt Washes

Apply **3 ml of High Salt Buffer** to the top of the resin and replace the top closure of the column. Resuspend the resin by gently mixing. This may require tapping the bottom of the column several times. Place the column in a 15 ml tube. Centrifuge at **350 × *g*** for **2 min**. Remove the top closure and discard the supernatant.

+ 3 ml of High Salt Buffer

Mix



Spin 2 min
350 × *g*
Discard supernatant

Repeat **two more times** for a total of 3 washes.

Repeat twice

Low Salt Washes

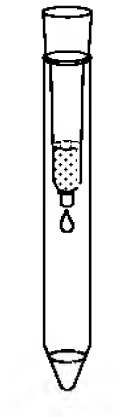
Apply **3 ml of Low Salt Buffer** to the column and replace the top closure. Resuspend the resin by gently mixing. This may require tapping the bottom of the column several times. Place the column in a 15 ml centrifuge tube. Centrifuge at **350 × *g* for 2 min**. Remove the top closure, discard the supernatant.

+ 3 ml Low Salt Buffer

Mix

Spin 2 min
350 × *g*
Discard supernatant

Remove the bottom closure. Apply **3 ml of Low Salt Buffer** to the top of the resin. Centrifuge at **350 × *g* for 2 min**. Remove column from centrifuge tube and discard the flow-through.



+ 3 ml Low Salt Buffer

Spin 2 min
350 × *g*

Discard flow-through

6. Elution

Place a sterile 1.5 ml screw-top microcentrifuge tube inside a 15 ml centrifuge tube. Place the column inside the 15 ml centrifuge tube in such a way that the tip of the column is inside the opening of the screw-top microcentrifuge tube (the collection tube).

+ 0.25 ml
Elution Buffer

For each elution, pipet **0.25 ml of prewarmed (65°C) Elution Buffer** onto the top of the column and centrifuge at **350 × *g* for 2 min**. Repeat elution another **two times** for a total of three elutions.

Spin 2 min
350 × *g*

Repeat twice

Do not change the collection tube between elutions. Collect the enitre 0.75 ml eluate in the same sterile tube.

Remove spin column from the centrifuge tube. Using sterile forceps, recover the microcentrifuge tube containing the column eluate.

Place the collected sample on ice. Proceed to Section 7.3. if a second column purification of the sample is desired.

7.2. Support protocol QuickPrep mRNA purification kit: Concentration of mRNA by precipitation

1. Prepare sample

Add **75 µl (1/10 volume) of Potassium Acetate Solution** (provided) and **10 µl of Glycogen Solution** (provided) to the **0.75 ml of eluted mRNA sample**. Add **1.5 ml of chilled 95% ethanol** (2–2.5 volumes) and incubate the sample at **-20°C for a minimum of 30 min**.

The amount of glycogen should remain constant regardless of sample volume.

2. Collect sample

Centrifuge at max. speed in a microcentrifuge at **4°C for 5 min**.
If the RNA is not to be used immediately, store it in this precipitated state (in ethanol) at -80°C.

3. Resuspend mRNA pellet

Decant the supernatant and invert the tube over a clean paper towel. Gently tap the tube on the towel to facilitate the removal of excess liquid. When all traces of ethanol are gone, redissolve the precipitated RNA in an **appropriate volume of Elution Buffer or RNase-free water.**

To determine the appropriate resuspension volume, consider the RNA concentration desired, the concentration before precipitation and the volume of the sample subjected to precipitation. However, the percentage of the RNA recovered after precipitation will depend on the total amount present. With 10 µg of RNA, for example, approximately 70% will be recovered. You may therefore wish to

redissolve the pellet in a volume 25–50% smaller than would be required if all of the RNA were recovered.

7.3. Support protocol QuickPrep mRNA purification kit: Second column purification

Prewarm the Elution Buffer to 65°C.

1. Prepare column

Invert an Oligo(dT)-Cellulose Spin Column **several times** to resuspend the resin.

Remove the top closure then the bottom closure. Place the column upright in a **15 ml centrifuge tube** and centrifuge at **350 × *g* for 2 min**. Remove the column from the centrifuge tube and discard the liquid in the tube. Replace the bottom closure on the drained column and place it upright in a rack.

2. Prepare sample

Use **Elution Buffer** to bring the final volume of the RNA samples to 1 ml. Incubate the sample at **65°C for 5 min** then chill on **ice for 5 min**.

Add **0.2 ml of Sample Buffer** and mix gently. Place on ice.

3. Purification

Apply the sample to the top of the column bed and allow it to enter the resin under gravity. Remove the bottom closure. Centrifuge at **350 × *g* for 2 min**.

4. Washes

<u>High Salt Washes</u>

Apply **0.25 ml of High Salt Buffer** to the column, then centrifuge at **350 × *g* for 2 min**. Repeat this wash step with an additional **0.25 ml** of High Salt Buffer and centrifuge again.

Low Salt Washes

Wash the column with **0.25 ml of Low Salt Buffer** and centrifuge at **350 × *g* for 2 min**. Repeat this **2 more times** for a total of three washes.

5. Elution

Place a sterile 1.5 ml microcentrifuge tube inside the 15 ml tube to collect subsequent eluates from the column. Replace the column with its tip inside the microcentrifuge tube.

To collect bound poly(A) RNA, elute **three times** with **0.25 ml of Elution Buffer**, prewarmed to 65°C. Centrifuge at **350 × *g* for 2 min** after each application and collect the entire **0.75 ml** eluate in the same sterile microcentrifuge tube.

Remove spin column from the centrifuge tube. Using sterile forceps, recover the microcentrifuge tube containing the column eluate. Keep tube and eluate on ice.

If the RNA is not to be used immediately, store it at -80°C. If RNA sample concentration is desired, see Section 7.2.

8. Appendix

8.1. Quantitation of mRNA

This spectrophotometric procedure will consume one third of the sample.

Procedure for pre-treating quartz cuvettes:
Soak 0.5 ml quartz glass cuvettes in a concentrated HCl:methanol solution (1:1) for 1 hr. Then rinse the cuvettes several times in RNase-free water.

For spectrophotometer quantitation, we recommend transferring the samples to microcentrifuge tubes and spinning at max. speed in a microcentrifuge for 1 minute to remove the residual cellulose resin. Carefully pipet the supernatant into a clean RNase-free microcentrifuge tube. Discard the original tube.

We suggest that you determine the concentration of the mRNA via spectrophotometry using undiluted eluate, placing the sample in a pretreated cuvette and recovering it for a downstream application. A spectrophotometer such as the NanoVue™ will help minimize the amount of mRNA sample necessary for this purpose. The absorbance reading at 260 nm must be between 0.05 and 2.0 to reflect the RNA concentration accurately. If the absorbance at 260 nm is > 2.0, the sample should be diluted to determine the RNA concentration. The minimum absorbance which can be relied upon to be accurate is 0.05, equivalent to an RNA concentration of 2 µg/ml.

Calculate the concentration of the RNA present in the eluate ([RNA]) using the standard formula (5):

$[RNA] = A_{260} \times D \times 40$ µg/ml
D = final dilution factor (in the simplest case above, this would be 1).

If the absorbance of the diluted sample is greater than or equal to 0.5, the sample may be used directly for cDNA synthesis utilizing the cDNA Synthesis Kit (27-9260-01), TimeSaver™ cDNA Synthesis Kit (27-9262-01), First-Strand cDNA Synthesis Kit (27-9261-01), or Ready-To-Go™ T-Primed First-Strand Kit (27-9263-01). If the absorbance is less than 0.5, the sample must be precipitated and redissolved at a higher concentration to ensure efficient cDNA synthesis utilizing any of these kits.

8.2. Composition of kit components

Table 8.1. Composition of kit components

Oligo(dT)-Cellulose columns	Suspended in storage buffer containing 0.15% Kathon™ CG/1CP Biocide
Extraction Buffer	Buffered aqueous solution containing guanidine thiocyanate and N-lauryl sarcosine
High Salt Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.5 M NaCl
Low Salt Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.1 M NaCl
Elution Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA
Sample Buffer	10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 3.0 M NaCl
Glycogen Solution	5–10 mg/ml glycogen in DEPC-treated water
Potassium Acetate Solution	2.5 M potassium acetate (pH 5.0)

8.3. References

1. Chirgwin, J.M. *et al.*, *Biochemistry* **18**, 5294 (1979).

2. Pharmacia P-L Biochemicals, *Analects* **17.4** (1989).

3. Pharmacia P-L Biochemicals, *Analects* **16.2** (1988).

4. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, second edition (1989).

5. Ausubel, F.M. *et al.* (Ed.), *Short protocols in molecular biology: a compendium of current protocols in molecular biology* (5th ed), John Wiley and Sons, Inc. Indianapolis, IN (2002).

8.4. Troubleshooting guide

Problem: Oligo (dT)–Cellulose resin leaking from column

Possible cause	Suggestions
Column design	• A small amount of Oligo (dT)–Cellulose resin will normally leak from the column during the column draining steps. Any resin that may leak will be removed during the draining and washing steps of the column preparation and will not affect the integrity of the mRNA sample.

Problem: The column drains slowly

Possible cause	Suggestions
Air bubble in frit	• Typically, a sample should drain through a column in 15–30 minutes. If it takes longer than 30 minutes, there may be an air bubble stuck in the frit of the column. If a sample does not drain, push the top cap back on the column. This will usually apply sufficient pressure to push any air bubbles out of the frit. To prevent air from being pulled back into the column, replace the bottom cap onto the tip of the column before removing the top cap. After the top cap has been removed, the bottom cap can be removed and the column should drip normally.
Viscosity of the sample	• If the viscosity of the sample is a problem, the column may drain slowly and then stop. The caps can be placed on the column and

Possible cause	Suggestions
Viscosity of the sample	the column gently rocked for 10–15 minutes to allow the sample to bind. The column can be centrifuged to clear it of the unbound material. Proceed with the protocol as written.

Problem: Low yield

Possible cause	Suggestions
Sample material	• Tissue samples that contain high amounts of connective tissue, yeast cells and certain plant tissues are known to be difficult to disrupt, and special care must be taken to ensure that the cells are thoroughly disrupted when extracting RNA.

Problem: Samples are not of sufficient purity

Possible cause	Suggestions
Overloaded columns	• Applying more than the maximum amounts of cells or tissue to the columns will result in a loss of purity as reflected by a decrease in the A_{260}/A_{280} ratio. • The resin will appear highly viscous and mucous-like when the sample is mixed in the column.
Sample material	• Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption.

9. Related products

RNAspin Mini RNA Isolation Kit	25-0500-70
RNAspin Midi RNA Isolation Kit	25-0500-73
RNAspin 96 RNA Isolation Kit	25-0500-74
QuickPrep *Micro* mRNA Purification Kit	27-9255-01
mRNA Purification Kit	27-9258-01
Oligo(dT)-Cellulose Type 7	27-5543-02
Cesium Trifluoroacetate	17-0847-02
TimeSaver cDNA Synthesis Kit	27-9262-01
CodeLink™ Expression Assay Reagent Kit	320012
Ready-To-Go RT-PCR Beads	27-9266-01
RT-PCR Master Mix	E78370
First-Strand cDNA Synthesis Kit	27-9261-01
Ready-To-Go You-Prime First-Strand Beads	27-9264-01
Ready-To-Go T-Primed First-Strand Kit	27-9263-01
dNTP Set	28-4065-51
RNAguard Ribonuclease Inhibitor (Human Placenta)	27-0815-01
AMV Reverse Transcriptase	E70041Y
M-MLV Reverse Transcriptase	E70456Y
Amersham Hybond™-N+	RPN119B
Rediprime™ II DNA labeling system	RPN1633
AlkPhos Direct™ labeling and detection system	RPN3692
Amersham Hyperfilm™ MP	RPN1677K
Amersham Hyperfilm™ ECL™	RPN2114K

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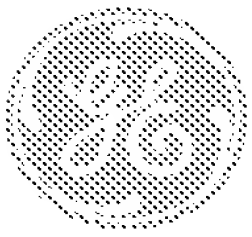

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Illustra™

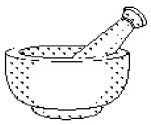
QuickPrep™ mRNA Purification Kit

Protocol reminder card 27-9258-01

mRNA purification from cultured cells and tissue

1. Sample homogenization

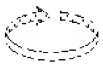
Tissue (< 0.5 g)

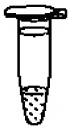


+ 1.5 ml Extraction Buffer

Homogenize

Cell culture (< 5×10^7 cells)

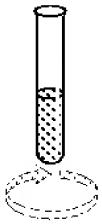




+ 1.5 ml Extraction Buffer

Transfer to tube

2. Cell lysis



+ 3 ml Elution Buffer
Mix

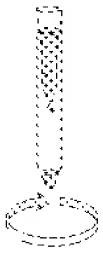
Spin 5-10 min,
12 000 × *g*

3. Prepare the Oligo (dT)-Cellulose spin column

Mix spin column resin
Remove top & bottom closures
Spin 2 min. 350 × *g*

Discard flow-through

4. Removal of cellular debris



+ 4 ml supernatant to resin
Mix 3 min

Spin 2 min. 350 × *g*
Discard supernatant

5. Washes		+ 3 ml of High Salt Buffer Mix
High Salt Washes		Spin 2 min, 350 × *g* Discard supernatant **Repeat twice**
Low Salt Washes		+ 3 ml Low Salt Buffer Mix Spin 2 min, 350 × *g* Discard supernatant
		+ 3 ml Low Salt Buffer
		Spin 2 min, 350 × *g* Discard flow-through
6. Elution		+ 0.25 ml Elution Buffer Spin 2 min, 350 × *g* **Repeat twice**

http://www.gelifesciences.com

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imagination at work

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